2-EEB-20

Reviewer: Richard C. Petrie

DRAFT

4/2/92

MRID No. 422624-02

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DATA EVALUATION RECORD

1. **CHEMICAL:** Ignite (glufosinate-ammonium). Shaughnessey No. 128850.

2. **TEST MATERIAL:** Ignite® Herbicide (Hoe 039866 00 SL18 A518); Batch No. C01594485; 18.3% active ingredient (as glufosinate ammonium); a blue liquid.

3. **STUDY TYPE:** Mollusc 48-Hour Embryo Larvae Study. Species Tested: Quahog Clam (*Mercenaria mercenaria*).

4. **CITATION:** Ward, G.S. 1992. Ignite® Herbicide (HOE 039866 00 SL18 A518): Acute Toxicity to Embryos and Larvae of the Hard Shell Clam (Quahog), *Mercenaria mercenaria* Under Static Test Conditions. Study No. J9105001b. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Hoechst Celanese Corporation, Somerville, NJ. EPA MRID No. 422624-02.

5. **REVIEWED BY:**

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Date: 6/29/92

6. **APPROVED BY:**

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7. **CONCLUSIONS:** This study is scientifically sound but does not meet the requirements for a mollusc embryo larvae study. No mortality data were included and the time between fertilization and test initiation was not reported. The 48-hour EC50 of 0.69 mg a.i./l (based on mean measured concentrations) classifies Ignite herbicide (formulated product) as highly toxic to quahog clams. The NOEC was <0.52 mg a.i./l (mean measured concentration).

8. **RECOMMENDATIONS:** See Section 14.D.(3).

9. **BACKGROUND:**

5.75

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10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Hardshell clams (*Mercenaria mercenaria*) were obtained from a supplier and maintained in unfiltered natural seawater for seven days prior to testing. The clams were periodically fed algae (*Isochrysis galbana*). The salinity of the seawater was 27-29 parts per thousand (ppt) and the temperature was 23.2-25.1°C.

Embryos were obtained by induced spawning of mature adult clams in test dilution water. Females were thermally induced in the presence of heat-killed sperm. Following release of the eggs, the female was removed from the chamber and live sperm were added. Fertilization was confirmed microscopically and estimated to be 100%.

B. **Test System:** The test chambers were covered 1-liter glass beakers containing 1 liter of test solution. The chambers were positioned in a temperature-controlled room (20 ±1°C) under a 16-hour light/8-hour dark photoperiod. Light intensity during the test was 766-1,300 ft-candles.

The dilution water was natural filtered (5 µm) seawater, adjusted to a salinity of 30 ppt, ultraviolet light sterilized, and filter-sterilized (0.45 µm). The stock solution (1,830 mg a.i./l) was prepared by diluting 0.9999 g of Ignite to 100 ml with deionized water. Appropriate volumes of the stock were mixed with dilution water to provide 3.5 l of each test solution.

C. **Dosage:** Forty-eight-hour static test. Based on the results of a preliminary test, six nominal concentrations (0.60, 1.0, 1.7, 2.9, 4.8, and 8.0 mg a.i./l) and a dilution water control were used.

D. **Design:** Seven milliliters of a clam embryo inoculum (3,600 embryos/ml) was added to each test chamber. Initial embryo densities in the test chambers were estimated by direct counts on the 4 control chambers using a Sedgewick-Rafter counting cell. At test termination, 4-ml samples from all chambers were collected and the number of normal and abnormal larvae in each sample enumerated. Each solution was mixed before sampling.

At test initiation, the dissolved oxygen concentration (DO) and pH of the prepared test solutions were measured. At test termination, DO and pH were measured in the individual replicates. The salinity of the dilution water control was measured at test initiation. The temperature of a control replicate was measured daily and the daily temperature range determined using a minimum/maximum thermometer.

Water samples from the control and exposure solutions were taken at test initiation and termination. Ignite herbicide concentrations were measured using high pressure liquid chromatography.

E. **Statistics:** The 48-hour median effective concentration (EC_{50}) and associated 95% confidence interval (C.I.) were calculated using probit analysis. Statistical differences in the number of normally developed clam larvae between the treatment and control groups were determined using analysis of variance (ANOVA) and Dunnett's test.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.52, 0.90, 1.54, 2.62, 4.22, and 7.12 mg a.i./l and ranged from 88 to 93% of nominal (Table 1, attached). Measured concentrations were generally consistent between sampling days.

After 48 hours of exposure, the mean number of normally developed larvae/ml ranged from 15.4 at 0.52 mg a.i./l to 0 at concentrations ≥4.22 mg a.i./l (Table 2, attached). The mean number of normally developed larvae/ml in the control was 20.3 which represents 16% control mortality ("i.e., 16 percent reduction in the number of clam larvae from initiation inoculum to test termination"). The 48-hour EC_{50}, based on mean measured concentrations, was 0.69 mg a.i./l (95% C.I. = 0.64-0.75 mg a.i./l). The no-observed-effect concentration (NOEC) was <0.52 mg a.i./l.

Dissolved oxygen concentrations were presented in Table 4 (attached). The pH values ranged from 8.3 to 8.5. The test temperature was 19.7-20.0°C. The salinity was 26 to 31 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author presented no conclusions.

A good laboratory practice statement was included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards

set forth in 40 CFR Part 160. The dates and types of quality assurance audits were also included.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with the SEP, except for the following:

The test design for a formulated product study should include a control where organisms are exposed to just the carrier and/or inert ingredients. Such a control was not included in this test.

The time between fertilization and test initiation was not reported. According to the SEP, tests should be initiated within one hour of fertilization.

Clam larvae survival must be reported and analyzed. Only mean survival in the control was reported and actual initial densities were not provided to allow the reviewer to calculate survival.

At test termination, the DO in the highest test solution was as low as 39% of saturation at 20°C and 31 ppt. The SEP states that DO must remain between 60 and 100% of saturation during the test.

The slope of the probit line was not given in the report.

Treatments should be randomly assigned to the test chambers. The author does not mention whether treatments were randomly assigned.

The recommended 15- to 30-minute dawn and dusk simulation periods were not used during testing.

B. Statistical Analysis: The reviewer used EPA's Toxanal program to calculate the 48-hour EC_{50} value as 0.70 mg/l (95% C.I. = 0.64-0.75 mg a.i./l) using the moving average method (see attached printout). The number of normal larvae/ml were not analyzed statistically since differences between the exposure groups and the control were visually evident (Table 2, attached).

C. Discussion/Results: Though the DO at the highest test level was less than 60% at test termination, the larvae were more likely affected by the herbicide since the next lowest concentration was equally affected and the DO was above 60% of saturation.

This study is scientifically sound but does not meet the requirements for a mollusc embryo larvae study. No mortality data were included and the time between fertilization and test initiation was not reported. The 48-hour EC50 of 0.69 mg a.i./l (based on mean measured concentrations) classifies Ignite herbicide (formulated product) as highly toxic to quahog clams. The NOEC was <0.52 mg a.i./l (mean measured concentration).

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental for a formulated product.

(2) **Rationale:** No mortality data were included and the time between fertilization and test initiation was not reported.

(3) **Repairability:** The study may be upgraded to "core" upon satisfactory review of the above data.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 06-03-92.

Table 1. Measured Concentrations of Ignite® Herbicide (Hoe 039866 00 SL18 A518) Active Ingredient During a 96-Hour Exposure of the Hard Shell Clam, (Mercenaria mercenaria) Under Flow-Through Conditions

Nominal Concentration (mg ai/L; ppm)	Measured Concentration (mg ai/L) 0 Hr[a]	48 Hr[b]	Mean	(±SD)	Percent of Nominal
Control	<0.018	<0.018	<0.018	-----	---
0.6	0.53	0.51	0.52	(0.01)	
1.0	0.90	0.89	0.90	(0.01)	90
1.7	1.55	1.52	1.54	(0.02)	90
2.9	2.64	2.61	2.62	(0.02)	90
4.8	4.42	4.03	4.22	(0.28)	88
8.0	7.10	7.13	7.12	(0.02)	89
1830 (stock)	1696	----	1696	(----)	93
SPIKE RECOVERY DATA					
MS (Rep A)	----	2.05	2.05	(0.00)	100
(Rep B)	----	2.05			

SD = Standard Deviation.

MS = Matrix spike. The matrix spike consisted of test substance in dilution water. The spike concentration was 2.04 mg ai/L and conducted in duplicate.

[a]All initial water samples were collected from replicate A test chambers.
[b]All final water samples were collected from replicate B test chambers.

Table 2. Number of Normally Developed Larvae of the Hard Shell Clam, Mercenaria mercenaria, Exposed to Ignite® Herbicide (Hoe 039866 00 SL18 A518), Under Static Test Conditions

Mean Measured Concentration (mg ai/L; ppm)	Rep	Number of Larvae/mL			Percentage Reduction[b]
		Abnormal	Normal	Mean[a]	
Control	A	0	20.3		
	B	0	22.7		
	C	0	18.4		
	D	0	19.7	20.3	----
0.52	A	0	15.1		
	B	0	16.3		
	C	0	14.8	15.4	24[b]
0.90	A	2.5	4.5		
	B	3.1	5.7		
	C	4.6	6.3	5.5	73[b]
1.54	A	0	0.3		
	B	0	0.6		
	C	0	0	0.3	98[b]
2.62	A	0	0.1		
	B	0	0		
	C	0	0	0.03	100[b]
4.22	A	0	0		
	B	0	0		
	C	0	0	0	100[b]
7.12	A	0	0		
	B	0	0		
	C	0	0	0	100[b]

[a] Mean values are the means of normally developed larvae.

[b] Significant ($P \leq 0.05$) reduction in number of normal larvae relative to the control.

Table 4. Dissolved Oxygen Concentrations During a 48-Hour Static Exposure of the Hard Shell Clam, Mercenaria mercenaria, to Ignite® Herbicide (Hoe 039866 00 SL18 A518)

Mean Measured Concentration (mg ai/L; ppm)	Replicate	DO Concentration (mg/L) Day 0	Day 2
Control	A	7.5	7.2
	B	---	7.1
	C	---	7.0
	D	---	7.1
0.52	A	7.7	7.2
	B	---	7.1
	C	---	7.2
0.90	A	7.7	6.9
	B	---	7.1
	C	---	7.1
1.54	A	7.7	7.1
	B	---	6.9
	C	---	6.8
2.62	A	7.7	6.6
	B	---	6.6
	C	---	6.5
4.22	A	7.7	6.2
	B	---	6.0
	C	---	5.9
7.12	A	7.7	3.9
	B	---	4.6
	C	---	4.4

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7.12	100	100	100	0
4.22	100	100	100	0
2.62	100	100	100	0
1.54	100	98	98	0
.9	100	73	73	0
.52	100	24	24	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .6968021

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
2	2.366731E-02	.698295	.6386581 - .7542698

.7542698

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RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	3.686111E-02	1	.9962909

SLOPE = 5.768496

95 PERCENT CONFIDENCE LIMITS = 4.660988 AND 6.876004

LC50 = .6948237

95 PERCENT CONFIDENCE LIMITS = .6428733 AND .7461195

LC10 = .4185172

95 PERCENT CONFIDENCE LIMITS = .3562454 AND .4699047

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Shaughnessey # 128850 Chemical Name IGNITE (glufosinate-ammonium) Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	18.3	EC_{50} = 0.69 ppm (95% C.L. product) (0.64 - 0.75) Control Mortality (%) = 16 Solvent Control Mortality (%) = N/A Species: Menenaria menenaria Slope = not given # Animals/Level = ~25,000/ml Temperature = 20°C Lab: Toxikon Environ. Sci. 48-Hour Dose Level ppm (% Effect) 0.52 (24), 0.9 (73), 1.54 (98), 2.62 (100), 4.22 (100), 7.12 (100) MRID # 422624-02 Comments: + mean measured concentrations	LMR 6/3/91	Supplemental
96-Hour EC_{50}		EC_{50} = pp (95% C.L.) Control Mortality (%) = Solvent Control Mortality (%) = Species: Slope = # Animals/Level = Temperature = Lab: 96-Hour Dose Level pp (% Effect) ( ), ( ), ( ), ( ), ( ) MRID # Comments:		